Diagnostics - Basic concepts of fish radiography

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Introduction

Radiography, or the use of X-rays for analysis, is the preferred method for fish skeletal deformity diagnostics. X-rays have enough energy to penetrate soft tissues, but not bone and other hard substances. Radiography thus allows the creation of a negative image of the skeletal structures of the fish, which allows the evaluation of the development and identification of pathology in the bones — without cutting into or even killing the fish. However, fish radiography is not a common procedure and, therefore, it represents some challenges for those who wish to use this diagnostic technique.

Small structures, weak contrast

The major limiting factors in fish radiography are **object size** and **low bone density** of the fish skeleton. These limitations are challenged constantly by the need or request for early diagnostics, leading to the sampling of very small fish.

In hatchery-size fish (i.e. juveniles), each structure of the skeleton is very small compared to standard radiography objects. The vertebrae in a salmon of 8 cm length will be less than 1 mm long; this is very small, even when compared to the bones in a kitten leg or other small mammalian objects. The mineral content of the developing fish bone is also low compared to mammalian counterparts. The consequence is that use of standard X-ray equipment, e.g. in a veterinary clinic, may produce images of too low contrast and/or resolution to be of any use for diagnostic procedures in small fish. In larger fish, on the other hand, such equipment can be more than adequate.

Mineral deposition in skeletal structures increases rapidly with fish age and size, after hatching and through first feeding. However, the time course and rate of mineralization also varies with species. Thus, even though an X-ray image of a 1g salmon may be of very little diagnostic value, an image of a sea bass of corresponding size may be fully adequate for evaluating skeletal deformities. It is therefore necessary to take both species and size into account when developing a diagnostic project based on radiography (see Figures 1a & 1b).

Figure 1a: Radiographic image of Atlantic salmon (Nofima Marin)

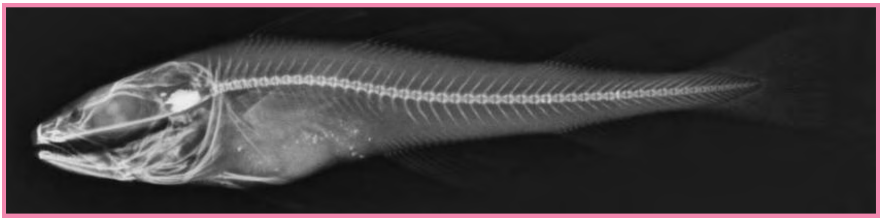

Figure 1b: Radiographic image of Atlantic cod (Nofima Marin)

The figures 1a and 1b are radiographic images of Atlantic salmon (1a) and Atlantic cod (1b), both of 1g size. The x-rays were taken with the same equipment and settings, illustrating the differences in development of skeletal structures between two species of the same weight (Photographs: Nofima Marin AS)

Analogue and digital radiography

Standard radiography images are made by exposure of a film, which is covered by a silver salt emulsion to X-rays. The image is subsequently created by developing the film. Undeveloped films are light sensitive, and development of the exposed film requires either a dark room or automated developer devices, as in traditional photography. When developed, the image exists on the film-foil only, and must be digitalized in a scanner or photographed in order to be processed further. These processes will commonly result in a certain loss of detail.

During the last decade, digital radiography has gradually taken over a significant part of the radiography market. Both direct digital systems and semi-digital systems are available. In direct digital systems, there are no films, and the image is recorded directly by a sensor. The image is subsequently created in the computer and displayed on a monitor. This technology does not yet give a good picture quality for fish, and it is still rather expensive. The semi-digital systems consist of a reusable image plate (substituting the film) and a plate reader, as well as the computer system. This system gives an image quality comparable to the traditional film-foil systems, even of small fish if one uses equipment used for mammography .

Both direct digital and semi-digital systems use the same type of radiography sources as analogue radiography.

The advantages of digital radiography, compared to analogue, are easier image storage and handling, resulting in images that can be subjected to analysis without loss of information. The digital systems may also provide automated procedures for image processing, e.g. contrast enhancement and edge sharpening.

Generally, digital systems also allow achievement of the same results - with lower radiation doses to the patient than in analogue radiography. This might not be so relevant for the fish but may still be of importance to the safety of the operator.

The main drawback of digital radiography compared to high quality analogue images is that you will get a limit in picture quality if you want to look closely at details, because a digital image is built up by pixels. Thus, the resolution is determined by pixel size.

There are also risks of creating artefacts in the computer, e.g. during contrast enhancement or as the result of unpredicted variation in greyscale. The use of automated image processing will also hamper the potential for comparing different groups of fish, since the image enhancement may treat different images in different ways. This variation is comparable to variation in image quality induced by the different settings (mAs, kV, film-focus distance) in analogue radiography.

X-ray sources

The challenge in working with small fish bones is that standard radiography systems give too strong radiation even at the lowest settings. Mammography, or soft tissue X-ray, was developed to detect small deviations in the density of human mammary gland tissue and, in mammography, special sources with low dosage are used.

Setups for mammography radiography, therefore, are generally much closer to the ideal for achieving X-rays of small fish, as opposed to standard equipment, and should be the preferred choice for any fish < 100g.

Mammography can also be used in bigger fish, eventually to image skeletal details when the body size limits imaging of whole fish (see Figure 2). In fish >100g, standard radiography will usually give acceptable results.

Figure 2: Mammography of four fused vertebrae in a 3kg Atlantic salmon. The level of detail is high, and the changes in the vertebrae adjacent to the fusion are clearly visible.
(Photo: Nofima Marin AS)

The difference in image resolution between standard radiography and mammography can be considerable; for instance, using 10 pixels/mm in a standard semi-digital system and 20 pixels/mm in a corresponding mammography system (Fuji Medical systems). This difference reflects a double reading (from both sides) of the mammography image plates, which are transparent on both sides of the absorbing material ("film"), compared to standard plates who are transparent on one side only.

Analogue radiography films also present a variety in film qualities, depending on the area of use. Mammography films are generally small in size but give good image quality with high resolution. Standard radiography films are delivered in several sizes designed for different body parts, and give a sufficient quality for mammalian structures and for bigger fish.

Technical x-ray is a tool designed for studying inanimate objects rather than animals, and has the potential of giving a very high image quality. This can be used for dead animals with good results, but is generally more expensive than standard films, and may require more adjustments to obtain the full potential from the film.

Mammography equipment is stationary, whereas standard X-ray sources come in a variety of different scales, also as mobile units that can be easily transported.

Standard radiography setups are commonly available in veterinary clinics and similar facilities. To find mammography equipment, it is usually necessary to identify human radiography laboratories, either in hospitals or clinics.

Settings and adjustments

There are three main settings to balance in order to obtain the best results, and for any setup, it will be necessary to adjust the equipment before use. It is strongly advised to engage a skilled radiography technician in this process.

1. kV (kilovolt) describes voltage
2. mAs (milliamp x seconds) electron current against the anode x exposure time in seconds.

   Note: The combination of kV and mAs defines the radiation dose.
3. The third variable is film-focus distance.
   In a typical standard radiography setup, film-focus distance can be adjusted within a wide range. In most mammography setups, the film-focus distance is fixed.

The relations in terms of the effects of radiation dosage is highly variable with different equipment, and it is difficult to provide universal guidelines.

These general considerations apply:

- Generally, fish radiography requires lower radiation doses (lower kV and mAs) than comparable setups for mammalian radiography in order to produce an acceptable image.
- Increased mAs gives less noise in the image and better contrast; an increase in mAs is a good option for improving image quality.
- Both increased kV and increased mAs give a darker image.
- Increased kV gives poorer contrast (greyer image) but better penetration (e.g. in thick(er) objects).
- Increased film-focus distance means that fewer x-rays reach the film since the rays spreads obliquely from the radiation source. Increasing this distance will allow you to have a larger exposure area (bigger film, more fish in one exposure), but the image quality may be somewhat reduced.

In the Nofima Marin fish X-ray laboratory, which is a semi-digital setup, common settings are 22 kV/50 mAs in mammography and 35 kV/50 mAs in standard X-ray.

Fish material for radiography

Fresh, newly killed fish is always preferable for radiography (Figure 3), but fish may be radiographed live or dead, fresh, frozen or fixed, and still give acceptable image quality.

Radiography is also less sensitive to sample freshness than most other analyses, as bones deteriorate much slower than soft tissues, and acceptable radiographs may be obtained of fish long after they have been rejected for other analytical purposes.

The best results are, however, obtained in fish that are sampled fresh and prepared for radiography. Ideally, and following a simple rule of thumb for radiography, fish should be placed sideways down, in a relatively straight posture.

When working with live fish for X-ray, sedation is necessary and must be sufficient to keep the fish still during exposure. For live radiography, it is important to sedate only a few fish at a time in a relatively strong sedative.

Use a few fish, strong sedation, short time in the air and then back to aerated clean water, rather than many fish, weak sedative and long time in the sedation.

The fish use oxygen from the water if they struggle for minutes before they become calm, thus the sedative solution soon becomes less efficient.

For fish welfare purposes, and to prevent unnecessary mortalities, it is strongly advised to pay sufficient attention to this point, and preferably have a designated and skilled person to handle the sedation.

Figure 3: Atlantic salmon (3.5 kg) euthanised and ready for radiography. Photo: Nofima Marin AS

For dead fish, it is equally important to pay attention to posture. It can be difficult to predict the onset of rigor mortis and, once the fish has entered rigor in a bent or skewed posture, it may be impossible to obtain a good image. If the fish is euthanised, it is still important to have aerated sedation and a sufficiently strong solution.

If there are too many fish in poorly oxygenated water, they will not be able to utilise the sedative because of lack of oxygen, and they will die from asphyxiation. In addition to the very poor welfare aspect of this, some of the fish will die gasping for oxygen. X-rays of gasping fish are useless for neck and, to some part, head diagnostics, and represent a visual disturbance to the evaluation in general.

Freezing

For X-ray purposes, fish must be frozen individually.

This is easily obtained by placing the fish side by side, in the right position, on a non-adherent, hard and smooth surface, e.g. a plastic covered board, and freeze in a standard freezer. When the fish are frozen through, they can be placed in plastic bags or boxes for storage.

Small fish <5g

Small fish will defrost quickly or can break even if they are well packaged.

This situation makes fixation of the fish specimens in 4% formaldehyde solution a better alternative. The fish are euthanised by an overdose of sedative and put into formalin solution. These are kept in the solution until properly fixed, when they get a “rubbery” consistence.

For histological purposes, the volume of fixative should be ten times that of tissue. If the fixation is for radiography only, a 5:1 ratio is sufficient.

The fish can then either be sent for analysis in the formalin solution in water proof containers, or rinsed in ethanol and sent in sealed plastic bags after removal of excess fluid.

Due to the known effects of local irritation and the possible carcinogenic effect of formalin, gloves should be used when handling the solution. A ventilation chamber is also necessary.

Large fish >1 kg

With large fish, the volume and weight alone can represent a challenge for freezing, handling and storage. Bigger fish can be filleted to save space, and to make transport easier and cheaper. Make sure, however, that the filleting knife does not cut into the spine. The spines can then be sent fresh on ice or frozen in a flat and straight position. Filleted spines on ice stays fresh for days and are easy to handle.

For evaluation purposes, it is a huge advantage that all fish are uniformly placed for imaging, e.g. all fish right side down, all dorsal fins towards the top, and all heads pointing to the left. This condition applies whether the material consists of single-fish images or if there are multiple fish per image.

Although having no effect on image quality per se, experience is that this simple measure eases the evaluation process and detection of deviations and differences between fish.

Labelling

Labelling is a commonplace, but very important issue if the fish are to be transported or stored, both for diagnostics and for experimental purposes. Confusion about the identity of the objects can make even the most perfect radiographic images worthless. The need for labelling may vary. In most cases, a group identity (ID) is sufficient, but in some cases there is a need to ID individual fish, which may be more challenging.

Groups can be labelled on the wrapping with a waterproof pen or pencil. It is a good rule to label groups very neatly - particularly if they are being sent to someone else; what may be logical to the sender may be completely confusing for the receiver. It is much better to write too much than not enough!

It is also important to remember that plastic bags may break when frozen, and that ink may dissolve if it gets wet, and to take due precautions. It is a good idea to duplicate the label information on a piece of paper, put it in a sealed plastic bag, and put it into the wrapping with the fish.

Tagging

There are numerous ways of tagging individual fish but only two are mentioned here:

1. Tagging of live fish with PIT-tags (see Figure 4). These tags are glass-encapsulated with a unique ID-code, and are placed in the abdominal cavity. This procedure is easily done on live, sedated fish > 10 grams. The tag remains in the abdomen for the entire life span of the fish, enabling identification with a PIT-tag reader. This tag type is commonly used for experimental purposes and in breeding programs, and allow for repeated retrieval of the same fish.

2. Dead fish can be labelled with a note of waterproof paper stapled to the tail fin (see Figures 4 and 5) or put under the operculum. Avoid putting notes in the fish' mouth as that forces the head back and the jaw open.

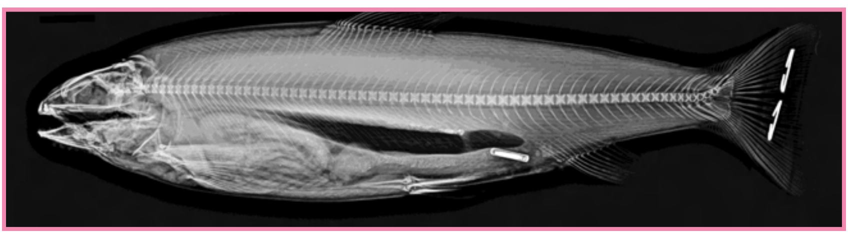

Figure 4. Atlantic salmon (150g) with PIT-tag in the abdomen (ID tag) and staples on the tail fin (external labelling). Radiograph taken by mammography. Photo: Nofima Marin AS

Figure 5: Salmon smolt euthanised, labelled and ready for freezing on a plastic covered board. Photo: Nofima Marin AS

Evaluation of radiographic images

The evaluation of fish radiographic images (Figure 6) is a science that remains under development. There are no reference laboratories for the evaluation of fish skeletal malformations, but numerous publications that describe skeletal pathology of different types and causalities exist. Also, in some sectors of aquaculture production, there is considerable competence within companies on how to read and interpret the images.

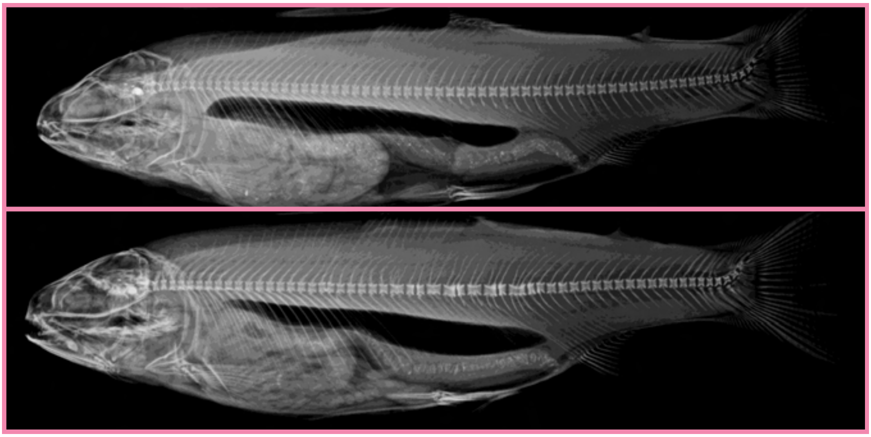

Figure 6. Rainbow trout (20 g) illustrating normal (top) and deformed (bottom) spinal columns. Radiograph taken by mammography. Photo: Nofima Marin AS

Guidelines for classification of skeletal malformations

Within the Fine Fish project, manuals for classification of skeletal deformities have been prepared for some of the most common aquaculture species.

- The combined sea bass and sea bream manual was prepared by the University of Patras (Greece), and presents extensive documentation of the most common malformations seen in these species, as well as current knowledge on causative factors.
- Manuals for Atlantic salmon, rainbow trout and Atlantic cod were compiled by the Nofima Marin (Norway) skeletal deformities research group.

Much less is published on skeletal malformations in these species, but these manuals provide examples and suggest some basic guidelines for classification.

These publications will be continuously updated. Please refer to the project website, www.finefish.info, for the latest versions of these manuals.